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THE METABOLISM OF FAT AND CARBOHYDRATE DURING HEMORRHAGIC SHOCK IN THE UNANESTHETIZED SUBHUMAN PRIMATE: CHANGES IN SERUM LEVELS OF FREE FATTY ACIDS, TOTAL LIPIDS, INSULIN AND GLUCOSE

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13. ABSTRACT

Five adult baboons were subjected to hemorrhagic hypotension for a period of 3-1/2 hours, and another five baboons served as the control animals. The animals were studied in a specially designed chair while fully awake. During the period of hypotension, the cardiac output decreased significantly from 3.2 ± 0.4 to 0.9 ± 0.2 L. per minute, and the arterial lactate rose from 16.4 ± 3.3 to 79.5 ± 19.9 mg. percent. Thirty minutes after hemorrhage, the serum insulin had fallen from 19.0 ± 0.1 to 7.6 ± 0.8 μU per milliliter, and the glucose had risen to a peak of 252 ± 20 mg. percent. Despite the development of hypoinsulinemia, no statistically significant acute change in the mean serum free fatty acid (FFA) concentration was demonstratable; however, after 3-1/2 hours the mean FFA level was 382 percent of base line in the hypotensive group and not significantly raised in the control animals. Possible reasons why the early hemorrhagic hypoinsulinemia did not lead to the expected early elevation in the FFA's are discussed.

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The metabolism of fat and carbohydrate during hemorrhagic shock in the unanesthetized subhuman primate: Changes in serum levels of free fatty acids, total lipids, insulin, and glucose

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Carbohydrate and fat are the major cellular substrates of oxidative metabolism; therefore, their fate during hemorrhagic hypotension is important to the understanding of the biochemistry of the low flow state. Previous work from this and other laboratories has shown that septic and hemorrhagic shock in the baboon are associated with hyperglycemia and hypoinsulinemia.[2, 3, 11] Other studies in experimental animals and patients have emphasized the mobilization and possible elevation of serum free fatty acids during stress and shock.[1, 3, 6, 8, 10, 12] The relationship between carbohydrate changes and lipid mobilization is closely linked in the normal subject; changes in this relationship during shock may be critical in understanding changes in cellular metabolism and the possible toxic effects of elevations of certain lipid moieties. Most of the previous work in this area has been carried out on anesthetized dogs; anesthesia and species variation may well make these results less applicable to the clinical situation. We have, therefore, undertaken the study of the relationship between fat and carbohydrate in the awake baboon subjected to hemorrhagic hypotension.

MATERIALS AND METHODS

Ten adult male baboons *(Papio doguera)*, weighing between 21 and 29 kilograms, were divided into two groups of five animals each. After an overnight fast of 10 to 12 hours, each animal was tranquilized intramuscularly with 1-(1-phenylcyclohexyl) piperidine hydrochloride (Sernylan), 1.0 mg. per kilogram of body weight, for insertion of catheters four hours prior to the start of the experiment. Polyethylene catheters were inserted into both femoral arteries and veins, and one venous catheter was threaded into the central position. The animal was then placed in a specially designed chair and allowed to awaken. One arterial and one venous catheter were connected to pressure transducers. By means of a preamplifier and

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The experiments reported herein were conducted according to the principles set forth in *Guide for Laboratory Animals Facilities and Care* prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

*Research Task No. M4305.05-3006AG02.

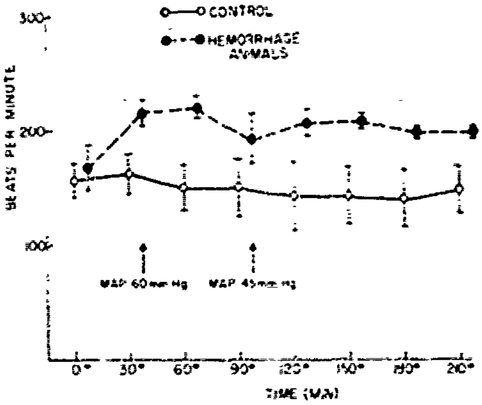

Fig. 1*A*. Heart rate changes in control and hemorrhaged baboons. MAP stands for the mean arterial pressure, as it does in all other figures in which it appears (mean ± S.E.M.).

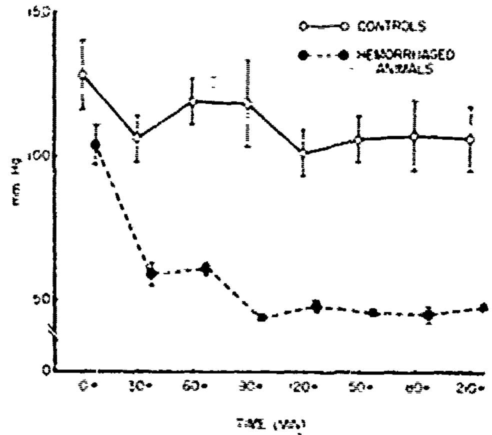

Fig. 1*B*. Mean arterial pressure changes in control and hemorrhaged baboons (mean ± S.E.M.).

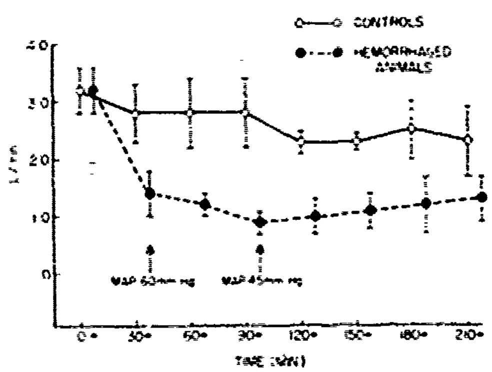

Fig. 1*C*. Cardiac output changes in control and hemorrhaged baboons (mean ± S.E.M.).

a multichannel polygraph with an oscilloscope, pressures were continuously monitored. Each pressure recording system was calibrated against a mercury manometer to insure linearity and accuracy. The electrocardiogram was also monitored continuously.

After base-line measurements were taken and blood samples were drawn, hemorrhagic shock was induced four hours after the baboon had received the Sernylan; at this point, the animal was fully awake. Blood was removed from the femoral artery catheter into a sterile bag containing acid-citrate-dextrose (ACD) solution. Blood was removed over a 15 minute period so that the mean arterial pressure would be reduced to 60 mm. Hg. This pressure was maintained for one hour; additional blood was then removed over a five-minute period until the mean arterial pressure was 45 mm. Hg. This final pressure was maintained for 3½ hours by withdrawal of more blood or infusion of some of the shed blood.

Cardiac output was measured every half hour during the experiment with the use of indocyanine green and a densitometer system. A known amount of dye was injected into the central venous catheter and arterial blood was aspirated for dye sampling by means of a withdrawal pump. After each determination, the blood was reinfused. The calibration factor for the densitometer was determined during the base-line measurement and at the end of the study. Arterial blood was drawn every 30 minutes for the following determinations: pH, Po_2, Pco_2, lactate, hematocrit, serum free fatty acids, serum total lipids, serum insulin, and serum glucose.

Arterial pH and gases were measured with the pH/gas analyzer* on blood which was collected anaerobically in heparinized glass syringes. Arterial lactate was determined by the enzyme method.† One to two milliliters of arterial blood was immediately deproteinized in a solution of perchloric acid and the supernatant was subsequently analyzed. Capillary tubes and a microcentrifuge were used to measure the hematocrit. For the fat analyses, the blood sample was immediately

*Instrumentation Laboratory, Inc., Boston, Mass.

†Lactate Stat-Pack, Calbiochem, Los Angeles, Calif.

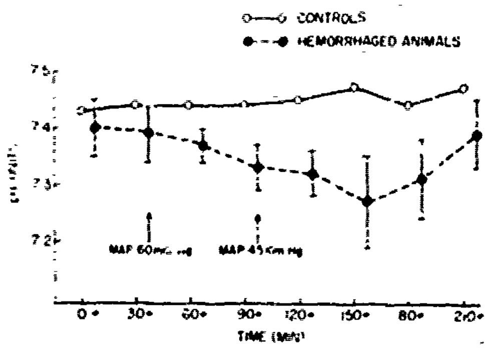

Fig. 2A. Changes in arterial pH during hemorrhagic shock in the baboon (mean ± S.E.M.).

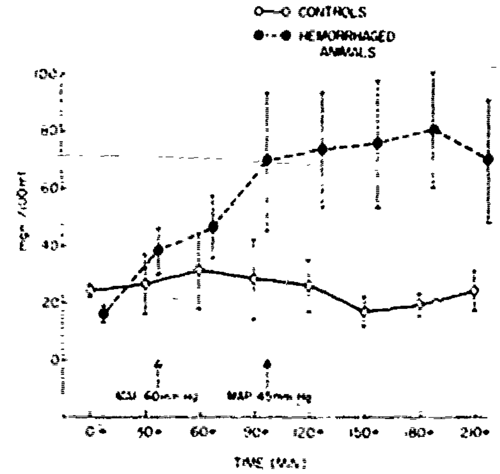

Fig. 2B. Changes in blood lactate level during hemorrhagic shock in the baboon (mean ± S.E.M.).

centrifuged and the serum was immediately removed and frozen for subsequent analysis. Free fatty acids were analyzed by the colorimetric micromethod of Mackenzie and co-workers.[9] A colored complex is formed between the free fatty acids, uranyl ion, and the basic dye, Rhodamine B; this complex is then measured in a spectrophotometer. Total lipids were measured by the colorimetric method based on the sulfo-phospho-vanillin reaction.[5] Serum insulin concentrations were measured by radioimmunoassay with the use of dextran-coated charcoal to separate antibody-bound insulin from unbound insulin.[7] Porcine insulin standards were used. Serum glucose levels were measured by the ferricyanide method on a dual-channel Technicon auto analyzer.

At the end of the experiment, one control animal and all five bled animals underwent autopsy; sections of lung, liver, and pancreas were removed for microscopic study. The mean, standard deviation, and standard error of the mean were calculated for all data; the statistical significance was determined with the Student's t test.

RESULTS

Hemodynamically, the control animals showed no changes in heart rate, mean arterial pressure, or cardiac output during the experiments (Figs. 1*A* and 1*B*). Likewise, the arterial pH and lactate did not change in the control animals (Figs. 2*A* and 2*B*). The hematocrit decreased from 38 ± 3 to 30 ± 4 percent (mean ± S.E.M.) over the three and one-half hour period, but this decrease was not statistically significant ($p > 0.1$). In the hemorrhaged animals, the heart rate did not change significantly during the experiment; however, the cardiac output decreased from 3.2 ± 0.4 to 1.4 ± 0.4 L. per minute ($p < 0.02$) 30 minutes after the onset of shock (Fig. 1*A*). The drop in cardiac output continued so that 30 minutes after the animal reached a mean arterial pressure of 45 mm. Hg, the output was 0.9 ± 0.2 ($p < 0.001$). At the end of 3½ hours, the output was 1.3 ± 0.4 ($p < 0.01$). The arterial pH tended to fall in the bled animals; however, this decline was never statistically significant (Fig. 2). The lactate level showed a significant rise 30 minutes after the onset of shock ($p < 0.05$); this increase continued throughout the experiment, resulting in a final value of 68.9 ± 20.6 mg. percent ($p < 0.05$) (Fig. 2). The hematocrit decreased significantly throughout the experiment; at 3½ hours, the value had fallen from 38 ± 2 to 26 ± 3 percent ($p < 0.02$).

The control animals showed no change in serum glucose or serum insulin levels during the experimental period (Figs. 3*A* and 3*B*). The shocked animals, on the other hand, showed a significant hyperglycemia of 252 ± 20 mg. percent 30 minutes after the onset of shock ($p < 0.02$). The serum glucose level in these animals returned to base line at the

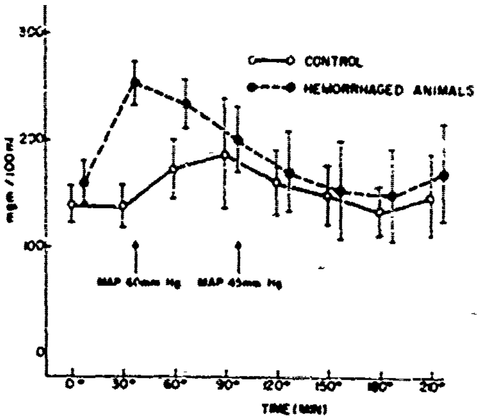

Fig. 3*A*. Serum glucose levels in control and hemorrhaged baboons (mean ± S.E.M.).

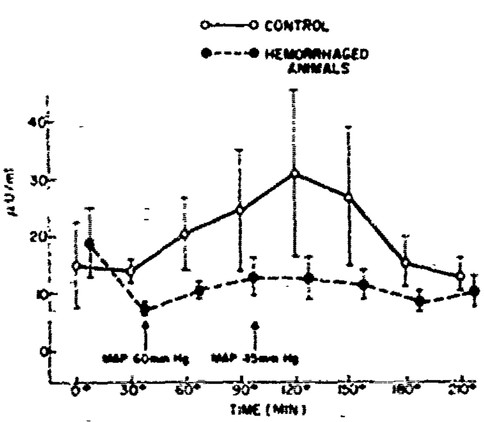

Fig. 3*B*. Serum insulin levels in control and hemorrhaged baboons (mean ± S.E.M.).

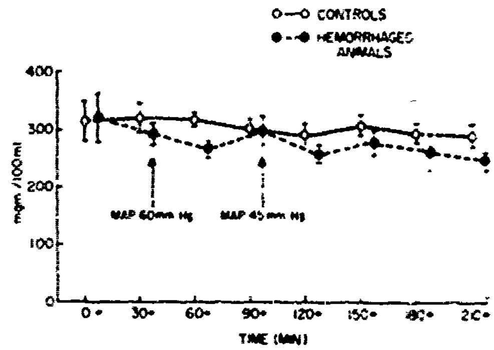

Fig. 4. Serum total lipids during hemorrhagic shock in the baboon (mean ± S.E.M.).

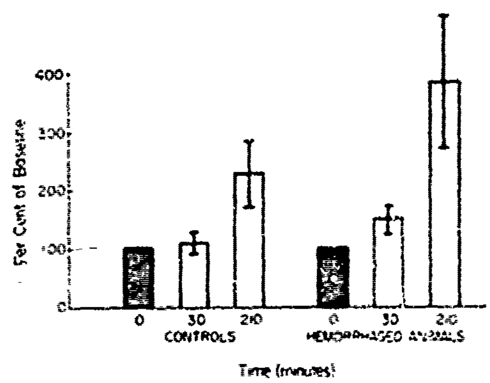

Fig. 5. Serum free fatty acids in control and hemorrhaged baboons. Percent change from base line (mean ± S.E.M.).

end of the study. Thirty minutes after hemorrhage, the serum insulin level had fallen from 19.0 ± 6.1 to 7.6 ± 0.8 μU per milliliter, significantly lower than the comparable control value ($p < 0.02$) (Fig. 3). Serum total lipids did not change in the control or the bled animals during the experiment (Fig. 4). The control animals showed no significant change in serum free fatty acids (FFA); however, 3½ hours after hemorrhage, the FFA level was 382 percent of base line ($p < 0.05$) in the shocked group, although no significant elevation was seen during the early hypotensive period (Fig. 5).

There were no significant gross or microscopic changes in the tissues from the control and bled animals. Of importance is the fact that no increased fat deposition was seen in the liver or lungs of the hemorrhaged animals.

DISCUSSION

The shock model used in the present studies causes a severe hemodynamic and metabolic insult to the animals, as evidenced by the marked decrease in cardiac output and the significant elevation in blood lactates. This model results in 100 percent mortality rate if the animal is not treated. Hypoinsulinemia along with hyperglycemia has been demonstrated in the hemorrhaged baboon by Moss and colleagues[11] and in the septic baboon by Cryer and co-workers.[2, 3] The relationship of these findings to changes in lipid metabolism, however, was not investigated.

Most of the investigations carried out on the effect of shock on serum FFA's and triglycerides have shown conflicting results. Farago and colleagues[4] demonstrated a decrease in serum FFA's during hemorrhagic shock in the dog. Groves and co-workers[6] and Kovack and colleagues[8] showed no change in FFA levels during hemorrhagic and septic shock in the dog. On the other hand, Carlson[1] showed that norepinephrine infusion led to fatty acid elevation in the serum of dogs, and Skillman and colleagues[12] demonstrated that a 15 percent bleed in normal humans led to a significant elevation of the level of the serum FFA's. Likewise, Mays[10] has shown that surgical trauma causes a significant elevation of serum FFA's. Our studies show that the severe stress of our hemorrhagic shock model causes an elevation of FFA's, but this rise becomes significant only 3½ hours after hemorrhage. One of the problems in studying the unanesthetized animal is the major degree of stress the baboon is subjected to before any shock is induced. This is reflected in the high base-line values of FFA's in the control and shocked animals (1.61 ± 0.47 and 1.85 ± 0.68 mEq. per liter). This stress also leads to a wide scatter in the data, making it difficult to demonstrate significant changes with small numbers of animals. The high base line of the FFA's was also seen in the study by Skillman and colleagues[12] on normal human volunteers, who were obviously stressed while various procedures were being carried out to perform the study; the initial values in their study were 1.36 mEq. per liter, a value much higher than the normal levels in humans. Other factors, which may totally or partially account for the lack of a statistically significant early rise in FFA's secondary to the early fall in serum insulin, are (1) a balanced increase in FFA mobilization and FFA utilization during hemorrhage and (2) rapid physiologic fluctuations in serum FFA levels which obscure small elevations during shock. In addition, the late rise of FFA's during hemorrhagic hypotension may reflect diminished FFA clearance due to decreased perfusion of metabolically active tissues.

The short period of investigation in the present study would preclude any possibility of detecting changes in total serum lipids, since changes in total lipids usually take several hours to days to occur. Groves and co-workers[6] found that the serum triglycerides rose in bacteremic dogs; however, they do not state at what time during the shock period this occurred (they studied their animals for 24 to 72 hours after the onset of shock).

SUMMARY

Five adult baboons were subjected to hemorrhagic hypotension for a period of 3½ hours, and another five baboons served as the control animals. The animals were studied in a specially designed chair while fully awake. During the period of hypotension, the cardiac output decreased significantly from 3.2 ± 0.4 to 0.9 ± 0.2 L. per minute, and the arterial lactate rose from 16.4 ± 3.3 to 79.5 ± 19.9 mg. percent. Thirty minutes after hemorrhage, the serum insulin had fallen from 19.0 ± 0.1 to 7.6 ± 0.8 μU per milliliter, and the glucose had risen to a peak of 252 ± 20 mg. percent. Despite the development of hypoinsulinemia, no statistically significant acute change in the mean serum free fatty acid (FFA) concentration was demonstratable; however, after 3½ hours the mean FFA level was 382 percent of base line in the hypotensive group and not significantly raised in the control animals. Possible reasons why the early hemorrhagic hypoinsulinemia did not lead to the expected early elevation in the FFA's are discussed.

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